A method for separating, extracting and purifying Poly- β - hydroxyalkanoates (PHAs) directly from bacterial fermentation broth

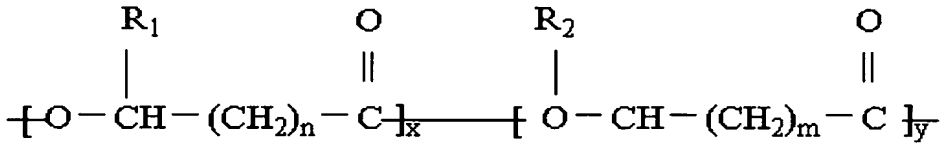
Technical field

This invention relates to post-treatment of biological engineering, particularly to extraction and separation of bacterial fermentation product, or more particularly to extraction and separation of polyhydroxyalkanoates in cells.

Background of invention

Poly- β -hydroxyalkanoates (PHAs) are biological polyesters accumulated in cells by special microorganisms under special growth conditions.

General formula:



In which, n and m are 1~4 integer, usually 1, that is 3-hydroxyalkanoates (3-HAS); R_1 and R_2 are straight chain or branched chain C_{1-12} alkyl which are substituted or non-substituted; X and Y are not 0 simultaneously, and determine the content of the component in copolymer. The average molecular weight of PHAs is generally 1-4 million Da.

The physical property of PHAs is similar to that of polypropylene. As its biodegradability, biocompatibility, piezoelectricity and optical activity are characteristics not possessed by common petrochemical resins, it has wide application prospect in industry, agriculture, medicine, sanitation, food, electronics, etc.

Large scale industrialized production of PHAs has not been realized internationally. The principal reason is the cost is much higher than that of petrochemical resin. The cost of PHAs includes mainly material cost and separation purification cost. The material cost depends on production efficiency of bacteria species and fermentation technology, whilst the separation purification cost depends largely on technology. The current extraction

technology includes separation of cells from fermentation liquid with high speed centrifuge and purification of PHAs in separated wet bacterial body with organic solvent extraction, chemical reagent or surfactant + enzyme. These methods have the defect of high cost or serious pollution, and are difficult to be industrialized. One step extraction separation method for extracting polyhydroxyalkanoates directly from fermentation liquid containing cells is disclosed in Chinese patent application CN1328160A, but it must use large quantity of sodium hypochlorite and has the defect of poor operation environment, serious pollution, cost increased by waste water treatment, and product quality affected by shear degradation of PHAs.

The purpose of this invention is to provide an extraction method for PHAs, which can reduce effectively separation and purification cost, reduces pollution, and is suitable for industrialized production.

Invention

This invention provides a method for directly separating and purifying polyhydroxyalkanoates in cells from bacterial fermentation liquid, comprisings:

(1) pretreating fermentation liquid with physical method for breaking cell wall;

(2) adjusting the pH value of the pretreated fermentation liquid so that it is alkaline;

(3) adding anionic surfactant and agitating;

(4) separating and extracting precipitate in reaction liquid;

(5) washing and drying.

The sequence of adjusting pH and adding surfactant is interchangeable.

In step 3, aside from adding anionic surfactant, coagulating agent can be added.

The physical method used to break cell wall can be ultrasonic breaking, ball milling or high pressure treatment.

The pH of the pretreated fermentation liquid is adjusted to 8-13. The alkaline substance used in adjusting pH can be solid or aqueous solution of NaOH, Na_2CO_3, $NaHCO_3$ or ammonia water.

The anionic surfactant can be olefinesulfonate (AOS), fatty alcohol sulfate, fatty alcohol polyoxyethylene-ether sulfate (AES), fatty alcohol-polyoxyethylene ether (AEO),

alkylphenol-polyoxyethylene ether, etc., its quantity is 0.5-20% (W/V) of fermentation liquid.

The coagulating agent is sodium polyacrylate, modified starch, polyamine, etc., its quantity is 0.5-20% (W/V) of the fermentation liquid.

After adding the anionic surfactant and the coagulating agent, the reaction temperature under agitation is 10-70℃ and the reaction time 5-60min.

Centrifuge, filter-press, vacuum suction filtration, etc. can be used for separating and extracting precipitate from the reaction liquid.

The invention is applicable to separation and purification of fermentation liquid of bacteria and its aberrance and gene engineering bacteria containing polyhydroxyalkanoates. Applicable bacteria include Alcaligenes, Pseudomonas, Azotobacter, Rhodospirillum, Methylotrophs, Bacillus, etc.

The invention has no high requirement for dry weight of cells and content of PHAs in fermentation liquid. The invention has the advantage of simple technology, low cost, high yield and greatly reduced pollution, so large scale industrialized production can be realized.

Detailed description of the invention

Following examples are used to further describe the invention. These examples should not constitute any limitation to the scope of claims. Any modifications or changes made by the skilled man in the art benefit from the disclosure of this application should be included within the scope of claims stated in this application.

Example 1

Take 1000ml of fermentation liquid of Alcaligenes entrophus mutant 65-7, in which the dry weight of cells is 142g/l, the content of PHBV is 78.5%; pretreat with ball mill (530r/min, 0.1mm steel ball) for 40min; adjust pH value to 12 with 30% NaOH solution; add 13g of sodium laurylsulfate; adjust reaction temperature to 32 ℃; react under agitation for 5min; filter with suction and filter paper; wash precipitate with water till washing becomes neutral; dry at 70℃ to constant weight. Purity of the product is 98.2%, the average molecular weight is 5.2×10^5Da, the yield is 85.2%. the COD and BOD of

waste water from suction filtration after treatment with anaerobic and aerobic bacteria is 800 and 30mg/l respectively, in conformity with state discharge standard.

Example 2

Take 100ml of fermentation liquid of Alcaligenes entrophus, in which the dry weight of cells is 147g/l , the content of PHBV is 75.2%; break cell wall with ultrasonic (1500W) for 20min; adjust pH value to 8 with 30% NaOH solution; add 0.5g of sodium laurylsulfate and 5g of sodium polyacrylate; adjust reaction temperature to 70℃; react under agitation for 30min; filter with suction and filter paper; wash coagulated precipitate with water till washing becomes neutral; dry in oven at 70℃ to constant weight. Purity of the product is 93.2%, the average molecular weight is $4.1x10^5$Da, the yield is 80.3%.

Example 3

Take 50ml of fermentation liquid of Alcaligenes entrophus, in which the dry weight of cells is 102g/l (in which the content of PHB is 60%); pretreat with ball mill (560r/min, 0.1mm steel ball) for 30min; adjust pH value to 13 with $NH_3.H_2O$ solution; add 10g of sodium laurylsulfate and 10g of modified starch; adjust reaction temperature to 10℃; react under agitation for 10min; separate with centrifuge (separation factor 600); wash precipitate with water till washing becomes neutral; dry in oven at 70℃ to constant weight. Purity of the product is 98.2%, the average molecular weight is $4.4x10^5$Da, the yield is 87%.

Example 4

Take 500ml of fermentation liquid of Alcaligenes entrophus mutant 65-7, in which the dry weight of cells is 135g/l, the content of PHB is 75.5% and introduce it into a special vessel. Increase pressure to 60MPa, release pressure rapidly after 10min, collect the liquid and repeat the operation twice. Adjust pH value to 10 with 30% NaOH solution; add 9g of sodium lauryl polyoxyethylene ether sodium sulfate; adjust reaction temperature to 38℃; react under agitation for 8min; filter with suction and filter paper; wash precipitate with water till washing becomes neutral; dry at 70℃ to constant weight.